REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*

2. REPORT DATE
April 25, 2002

3. REPORT TYPE AND DATES COVERED
Final Report - April 1 1996 - Dec. 31, 1999

4. TITLE AND SUBTITLE
Fate of Complex Aromatic Petroleum Hydrocarbons in Marine Sediments: Biological Transformation, Degradation and Sequestration

5. FUNDING NUMBERS
N00014-98-WX-20172

6. AUTHOR(S)
Sabine E. Apitz, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
SPAWAR Systems Center, San Diego D361
53475 Strothe Road
San Diego, CA 92152-6325

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217-5000

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
—

20020517 030

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution Unlimited

13. ABSTRACT *(Maximum 200 words)*

The objective was to develop methods to determine how low-level mixtures of PAHs degrade aerobically in marine sediment systems, in order to determine what rate and extent of biodegradation can be expected in marine sediments under natural and engineered conditions. Patterns of PAH biodegradation were evaluated under various conditions. The relative and absolute losses of mixtures of PAHs under various experimental conditions were compared. Novel methods were developed and validated to synthesize "realistic" model sediments for biodegradation and fate experiments. A number of conclusions can be drawn from experimental results: 1) Even "aged" model samples behave in a less complex way than do field-contaminated samples, but they still provide insight into trends. 2) Mineralization experiments underpredicted ***extent*** of attenuation, relative to experiments which track loss of extractable PAH, but they did show degradation ***potential***. 3) Much of extractable PAH loss observed in the laboratory and field is most likely not mineralization, but rather is probably the result of binding and transformation processes. 4) 3-5 ringed PAHs appear sensitive to degree of aeration, but do attenuate impressively under realistic surface conditions (i.e., occasionally aerated by natural processes). 5) Laboratory results should be extrapolated to the field with caution.

14. SUBJECT TERMS
biodegradation, PAH, marine sediments, model systems, irradiation, PAH degrader

15. NUMBER OF PAGES
5 (including this page)

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	UL

GRANT #: N00014-98-WX-20172

PRINICIPAL INVESTIGATOR: Dr. Sabine E. Apitz

INSTITUTION: Space and Naval Warfare Systems Center, San Diego, 2361

GRANT TITLE: Fate of Complex Aromatic Petroleum Hydrocarbons in Marine Sediments: Biological Transformation, Degradation, and Sequestration

AWARD PERIOD: April 1, 1996 - December 31, 1999

OBJECTIVE: The objective was to develop methods to determine the factors that control how low-level mixtures of PAHs degrade aerobically in marine sediment systems, in order to determine what rate and extent of biodegradation can be expected in marine sediments under natural and engineered conditions.

APPROACH: Patterns of PAH biodegradation were evaluated under various conditions. The relative and absolute losses of mixtures of PAHs under various experimental conditions were compared. Novel methods were developed and validated to synthesize "realistic" model sediments for biodegradation and fate experiments. The hydrodynamics of low-cost, low-energy, high capacity bioreactors were examined.

ACCOMPLISHMENTS: 1) The capacity of San Diego Bay microbial consortia to degrade or attenuate selected PAHs (phenanthrene, fluoranthene, pyrene, chrysene, and benzo(a)pyrene) in marine sediments was examined using a variety of laboratory techniques. Results suggested that even recalcitrant and sequestered compounds such as benzo(a)pyrene can be affected by microbial activity, but that oxygen availability can be a controlling factor in the patterns of degradation. Clearly, how degradative capacity is examined also has a significant effect upon what is observed. 2) The fate of polycyclic aromatic hydrocarbon (PAH) mixtures in marine sediments can be difficult to determine due to extraction, analytical and matrix barriers. The purpose of this work unit was to develop and validate methods to kill indigenous microorganisms in marine sediments, to spike the sediments with a mixture of PAHs in a minimally invasive fashion and to age the treated sediments while following the effects of the treatments on the PAHs and several groups of microorganisms. Following gamma irradiation (0.0, 2.5, 3.5 and 5.0 MRad), the sediments were mixed with known amounts of PAHs that had been coated onto fine-grained sand. During the subsequent aging process, levels of extractable PAHs and numbers of microorganisms were monitored. The addition of PAHs to the unirradiated sediment seemed to rapidly induce the degradation of phenanthrene, fluoranthene and pyrene, while these PAHs decreased to a much smaller extent in the irradiated sediments in the 376 days of the experiment. The heavier PAHs, chrysene and benzo(a)pyrene, showed slight decreases in extractable concentrations at all irradiation levels, suggesting PAH sequestration/aging over time. Using the methods described, a library of killed, labelled/spiked marine sediments was generated. 3) Changes in indigenous bacterial populations were monitored in the above experiments. While phenanthrene- and chrysene-degraders were present in the unspiked sediments, and increased during handling, PAH-spiking of non-irradiated sediments led to dramatic increases in their numbers. Phenotypic characterization of isolates able to grow on phenanthrene or chrysene placed them in several genera of marine bacteria: Vibrio, Marinobacter or Cycloclasticus, Pseudoalteromonas, Marinomonas and Halomonas. ***This is the first time marine PAH-degraders have been identified as the latter***

two genera, expanding the diversity of marine bacteria with this ability. Even at the highest irradiation dose (10 Mrad), heterotrophs and endospore-formers reappeared within weeks. However, while bacteria from the unirradiated sediments had the capacity to both grow on and mineralize 14C-labeled phenanthrene and chrysene, irradiation prevented the reappearance of PAH degraders for up to 4 months, allowing spikes to age onto the sediments, which can be used to model biodegradation, availability and fate in marine sediments. 4) Hydrodynamics and mass transfer characteristics of a three-phase airlift reactor were studied in a rectangular split-vessel reactor and using an air-seawater-marine sediment system. Experiments were conducted for two-phase systems using marine sediments. The presence of fine sediment particles in the system had little effect on hydrodynamic and mass transfer parameters up to 25% loading. The airlift reactor was found to meet the dissolved oxygen demand needed for a contaminated sediment treatment process.

CONCLUSIONS: If intrinsic or enhanced biodegradation of PAHs in marine sediments is to be part of a contaminated sediment management strategy, it is critically important that we determine the degradative potential of native microbial populations. However, translating information about what microbes *can* do (e.g., the behavior of an isolate in a laboratory fed a sole carbon source) to what microbes *will* do in the field (e.g., complex consortia exposed to a trace contaminant in a complex, carbon-rich matrix) is an inexact science. If we are to use these experiments to "predict" attenuation, a number of conclusions can be drawn: 1) Even "aged" model samples behave in a less complex way than do field-contaminated samples, but they still provide insight into trends. 2) Mineralization experiments underpredicted ***extent*** of attenuation, relative to experiments which track loss of extractable PAH, but they did show degradation ***potential***. 3) Much of extractable PAH loss observed in the laboratory and field is most likely not mineralization, but rather is probably the result of binding and transformation processes which are not yet completely understood. 4) 3-5 ringed PAHs appear sensitive to degree of aeration, but do attenuate impressively under realistic surface conditions (i.e., occasionally aerated by natural processes). 5) Laboratory results should be extrapolated to the field with caution.

SIGNIFICANCE: The methodologies for generating well-characterized, labeled model sediments, as well as data on patterns of degradation of PAHs under various experimental conditions, are powerful tools for examining the critical fate, mass balance and bioavialability questions which must be addressed to translate work on what marine microbes *can* do to information about what they *will* do in real systems. In our experiments, significant attenuation of many PAHs was observed under most conditions, but much remained when degradation rates leveled off (in the classic "hockey stick" shaped pattern). All experimental systems that seek to reflect complex natural processes must strike a balance between "realism" and control. Because of choices made to maximize realism in these experiments, it is unclear how much of the PAH losses observed in static samples resulted from original aeration during homogenization, and how much happened anaerobically. While generally anaerobic within millimeters, many marine sediments are periodically aerated, via resuspension, bioturbation, etc. Current advances in observing the benthic interface using time-lapse Sediment Profile Imaging and 2-dimensional oxygen optrodes, as well as observations on porewater dynamics in micro-flow environments make clear just how dynamic surface sediments are, and seriously undermine

the image of a static, buried, anoxic system. Our laboratory results on PAH responses to aeration, combined with ONR-funded work demonstrating anaerobic capacity, as well as biomarker work and field observations of PAH patterns in disturbed vs. quiescent sediments suggest that there is strong evidence that PAH-contaminated sediments will continue to recover long after deposition. Based upon current theory on "irreversible sorption" of contaminants on marine sediments, and experimental results, it is possible that the "hockey stick" pattern observed in these and most other laboratory biodegradation experiments will be repeated indefinitely if sediments are periodically disturbed in situ. A modeling paper addressing this issue, and its implications for the prediction of the ultimate extent of natural attenuation, is being prepared with Prof. Louis Thibideaux of LSU. Further experimental investigation of these issues requires the use of labeled, spiked, aged sediments, such as those developed in this study.

PATENT INFORMATION: None.

AWARD INFORMATION: Promoted to DP-IV (GS 14/15)

PUBLICATIONS AND ABSTRACTS (for total period of grant):

1. S E Apitz (1997) "Biodegradation of PAHs in Field-Contaminated Sediments: Complications and Implications", *18th Annual SETAC Meeting, San Francisco, CA, Nov. 16-20,* 1997, p. 54.

2. S E Apitz, E W Lin, R J Melcher, B B Hemmingsen (1998) "Synthesizing a Realistic PAH-Spiked, Aged Marine Sediment: Trade-Offs and Observations" *Abstracts of the Am. Chem. Soc.*

3. R Melcher, E Lin, S Apitz, and B Hemmingsen (1998) "Construction of a Model System to Study Phenanthrene and Chrysene Degradation in San Diego Bay Sediments", 98th Annual ASM Meeting, Atlanta GA, May 21, 1998.

4. J Molinari (1998) "Characteristics of radiation resistant endospore-forming bacteria isolated from shallow marine sediments". "Annual Undergraduate Poster Competition, College of Sciences, and Student Research Symposium", sponsored by the Howard Hughes Biology Education Initiative, SDSU. April 16, 1998 (student is working with endospore formers isolated in irradiation experiments funded by work)

5. E W Lin, E Arias, S A Clawson and S E Apitz (1999) "The Recovery of Native Sediment Microbial Populations After Irradiation, Aging, and Reinoculation - Implications for Controlled Biodegradation Studies". ASM 99th General Meeting, Chicago, IL, May 30-June 3 1999.

6. M Tobajas, E Garcia-Calvo, M H Siegel and S E Apitz (1999) "Hydrodynamics and mass transfer in a three-phase airlift reactor for marine sediment biotreatment", *Chem. Eng. Sci.* 54, 5347-5354 (partial support).

7. E Lichtfouse, S E Apitz and M Nanny, Guest Editors (1999) "The Biogeochemistry of Polycyclic Aromatic Hydrocarbons: Sources, Interactions, Biodegradation, Toxicity and Analytical Developments"; Proceedings of a Symposium held at the Spring 1998 Nation Meeting in Dallas of the ACS, *Organic Geochemistry,* 30, v-970.

8. S E Apitz, E Arias, S A Clawson, E W Lin, R J Melcher and B B Hemmingsen (1999) "The Development of a Sterile, PAH-Spiked, Aged Marine Sediment for Biodegradation Experiments: Chemical Results", *Organic Geochemistry* 30, 891-900.

9. M Tobajas, M Siegel and S E Apitz (1999) "Influence of Geometry and Solids Concentration on the Hydrodynamics and Mass Transfer of a

Rectangular Airlift Reactor for Marine Sediment and Soil Bioremediation", *Canadian Journal of Chemical Engineering* 77, 660-669 (partial support).

10. R J Melcher, E W Lin, S E Apitz and B B Hemmingsen (1999) "Demonstration of biodegradation in PAH-spiked marine sediments: population changes and characterization of phenanthrene- and chrysene-degrading bacteria", ASM 99th General Meeting, Chicago, IL, May 30-June 3, 1999.

11. S E Apitz (1999) "PAH Biodegradability: Lessons Learned Using Model and Field-Collected Sediments", In situ and On-Site Bioremediation: at The Fifth International Symposium, San Diego, CA, April 19-22, 1999 (invited).

12. R J Melcher (1999) *Demonstration of biodegradation in polycyclic aromatic hydrocarbon-spiked marine sediments: population changes and characterization of phenanthrene- and chrysene-degrading bacteria.* M.S. Thesis, San Diego State University, San Diego, CA. 109pp.

13. S E Apitz, E Arias, S A. Clawson and E W. Lin (2000) "Biodegradation of mixed 3-5 ring polycyclic aromatic hydrocarbons in marine sediment: importance of aging, microbial pre-exposure and method selection", SETAC 21st Annual National Meeting, Nashville, TN, November 2000 (invited).

14. S E Apitz, E Arias, S A Clawson and E W Lin (2000) "Model Systems to Examine the Fate of mixtures of PAHs in Marine Sediments", Abstracts of the Second International Conference on the Remediation of Chlorinated and Recalcitrant Compounds, Monterey, CA, May 22-25, 2000 (invited).

15. M T Togna, J Kazumi, S E Apitz, V Kirtay and L Y Young (2001) "Effect of Sediment Toxicity on Anaerobic Microbial Metabolism", *Environmental Toxicology and Chemistry*, Vol. 20 No. 11: 2406-2410.

16. S E Apitz, E Arias and S A Clawson (2001) "Behavior of 3-5 Ring PAHs in San Diego Bay Sediments: Implications", *International Conference on Remediation of Contaminated Sediments*, October 10-12, 2001, Venice, Italy (invited).

17. S E Apitz, E Arias and S A Clawson (2001) "Behavior of 3-5 Ring PAHs in San Diego Bay Sediments Under Various Experimental Conditions: Implications for the Prediction of Natural and Engineered Attenuation", The Association for Environmental Health and Sciences 11th Annual West Coast Conference on Contaminated Soils, Sediments and Water, San Diego, CA, March 2001 (invited).

18. R J Melcher, S E Apitz and B B Hemmingsen (2002) "The impact of PAH-spiking, irradiation and aging on marine sediment microbial populations", (in press, *Applied and Environmental Microbiology*).

19. S E Apitz (manuscript) "Biodegradation of mixed 3-5 ring polycyclic aromatic hydrocarbons in marine sediment: importance of aging, microbial pre-exposure and method selection".

20. S E Apitz and L Thibideaux (in prep) "Irreversible sorption, relaxation and "hockey stick" biodegradation - monitoring natural recovery of PAHs in marine sediments".